Strep-Tactin Spin Column Purification Protocol

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1 The *Strep*-tag®/*Strep*-Tactin® system

The *Strep*-tag II is a short peptide (8 amino acids, WSHPQFEK), which binds with high selectivity to *Strep*-Tactin, an engineered streptavidin. The binding affinity of *Strep*-tag II to *Strep*-Tactin (K_d = 1 µM) is nearly 100 times higher than to streptavidin. *Strep*-tag can be genetically fused upstream or downstream to the reading frame of any gene and expressed as fusion peptide. This technology allows one-step purification of almost any recombinant protein under physiological conditions, thus preserving its bioactivity. The *Strep*-tag system can be used to purify functional *Strep*-tag II proteins from any expression system including baculovirus, mammalian cells, yeast, and bacteria.

After application of up to 600 µl of crude extract on a spin column and binding of the *Strep*-tag proteins on the *Strep*-Tactin matrix, unspecific proteins are washed away in four short washing steps. Gentle elution of up to 150 µg purified recombinant protein in a total of 50-450 µl is then performed by addition of the same washing buffer containing low concentrations (2 mM) of D-biotin.

The *Strep*-tag/*Strep*-Tactin interaction is compatible with a variety of reagents (see table 1) making the system attractive for purifying metallo- and membrane proteins, large proteins and protein complexes.

Because of its small size, *Strep*-tag generally does not interfere with the bioactivity of the fusion partner. Thus, removal of the tag becomes superfluous. Comprehensive reviews and scientific publications giving an overview of various *Strep*-tag applications are listed at www.iba-go.com.

General remarks

- The affinity matrix in the *Strep*-Tactin spin columns has the same purification properties as *Strep*-Tactin MacroPrep®. To enable small elution volumes containing the recombinant protein in high yield and high concentration, the spin column elution buffer contains biotin instead of desthiobiotin. Thus, *Strep*-Tactin spin columns cannot be re-used and this elution buffer should not be used with other Strep-Tactin resins.
- Since protein purification is based on the highly selective binding of *Strep*-tag to *Strep*-Tactin, the contact time of the lysate with the resin has to be sufficient for complete complex formation. Therefore, it is important not to exceed 700 x g (approx. 2000 rpm in a microfuge) when centrifuging *Strep*-Tactin spin columns for protein binding. However, washing steps should be performed at maximum speed.
- To ensure buffer flow during the centrifugation steps, the spin columns should be centrifuged with an open lid. For very viscous cell lysates, it may be necessary to extend the centrifugation time.
- The pH of all lysates and buffers should not be lower than 7.5.
- To prevent proteins from being degraded during cell harvest, lysis, or even purification, it is recommended to work quickly at 4°C and – if necessary – to add protease inhibitors.

Reagent	Concentration
Reduction Agents	
DTT	50 mM
β-mercaptoethanol	50 mM
Non-Ionic Detergents	
C_8E_4 Octyltetraoxyethylene	max. 0.88 %
$C_{10}E_5$; Decylpentaoxyethylene	0.12 %
$C_{10}E_6$	0.03 %
$C_{12}E_8$	0.005 %
$C_{12}E_9$; Dodecyl nonaoxyethylene (Thesit)	0.023 %
DM; Decyl-ß-D-maltoside	0.35 %
LM; N-dodecyl-β-D-maltoside	0.007 %
NG; N-nonyl-β-D-glucopyranoside	0.2 %
OG; N-octyl-β-D-glucopyranoside	2.34 %
TX; Triton X-100	2 %
Tween 20	2 %
Ionic Detergents	
N-lauryl-sarcosine	2 %
8-HESO;N-octyl-2-hydroxy-ethylsulfoxide	1,32 %
SDS; Sodium-N-dodecyl sulfate	0.1 %
Zwitter-Ionic Detergents	
CHAPS	0.1 %
DDAO; N-decyl-N,N-dimethylamine-N-oxide	0.034 %
LDAO; N-dodecyl-N,N-dimethylamine-N-oxide	0.13 %
Others	
Ammonium sulfate $(NH_4)_2SO_4$	2 M
$CaCl_2$	max. 1 M
EDTA	50 mM
Ethanol	10 %
Guanidine	max. 1 M
Glycerol	max. 25 %
Imidazole	max. 250 mM
$MgCl_2$	1 M
NaCl	5 M
Urea	max. 1 M

Table 1. Reagents compatible with the *Strep*-tag/*Strep*-Tactin interaction

Note: These reagents have been successfully tested for the purification of e.g. GAPDH-*Strep*-tag with concentrations up to those mentioned. For reagents not marked with "max." higher concentrations may be possible, though. Since binding depends on the sterical accessibility of *Strep*-tag in the context of the particular protein the possible concentration may deviate from the given value for other proteins.

2 Preparation of Cleared Lysates

Material and important notes

- Buffer W: 100 mM Tris/HCl pH8, 150 mM NaCl, 1 mM EDTA
- Buffer P (for the release of the periplasmic content after periplasmic expression: 100 mM Tris/HCl pH8, 500 mM sucrose, 1 mM EDTA
- It is recommended not to work with EDTA in case of metalloproteins
- 2 mg/ml polymyxin B sulfate may be used instead of EDTA for the release of the periplasmic content in case of metalloproteins
- 5x SDS-PAGE sample buffer: 0.25 M Tris·Cl pH 8.0; 25% glycerol; 7.5% SDS, 0.25 mg/ml bromophenolblue; 12.5% v/v mercaptoethanol

2.1 Preparation of cleared lysate after cytoplasmic expression of *Strep*-tag fusion proteins

1. **Chill Buffer W at 4°C. Resuspend the cell pellet of a 100 ml culture in 1 ml Buffer W.**

2. **Take a 10 µl sample for analysis of the total protein content via SDS-PAGE and/or Western blotting.**

   The 10 µl sample should be thoroughly mixed with 90 µl Buffer W and 25 µl of 5x SDS-PAGE sample buffer. The sample should be incubated in an ultrasonic bath for 15 minutes to break up chromosomal DNA and should be heated at 70°C for 10 minutes prior to SDS-PAGE.

3. **Sonicate the residual suspension under ice-cooling.**

   Take care that the suspension does not become warm or even hot which may denature proteins or activate proteases. Perform bursts under cooling. French pressing is possible as well. Lysis should be complete and can be determined by measuring the optical density at 590 nm [% lysis = $(1 - A_{590}^{sonicate}/A_{590}^{suspension}) \times 100$].

4. **(Optional) If the lysate is very viscous, add RNase A (10 µg/ml), DNase I (5 µg/ml) and $MgCl_2$ (5 mM) and incubate on ice for 10 – 15 min.**

5. **Centrifuge the suspension at 13000 rpm (microfuge) for 15 minutes at 4°C.**

6. **Carefully transfer the clear supernatant to a clean tube.**

   For analysis of the insoluble part of the expressed protein, dissolve the sediment with 1.25 ml 1x SDS-PAGE sample buffer (= 250 µl 5x SDS-PAGE sample buffer mixed with 1 ml Buffer W).
   Store the supernatant on ice until chromatography or store at -20°C if chromatography cannot be performed the same day.

2.2 Preparation of cleared lysate after periplasmic expression of *Strep*-tag fusion proteins

Periplasmic proteins are secreted into the periplasmic space located between the outer and inner membrane of *E. coli*. Proper secretion is only possible when the recombinant protein is fused to a N-terminal signal peptide (e.g. OmpA) which is cleaved following translocation by *E. coli* leader peptidase. In order to purify proteins secreted into the periplasmic space using immobilized *Strep*-Tactin, the *Strep*-tag can be fused to the C- or N-terminus using pASK-IBA2, 2C, 4, 4C, 6, 6C, 12, 14, 44.

1. **Chill Buffer P at 4°C.**

2. **Resuspend the cell pellet of a 100 ml culture in 1 ml Buffer P.**

3. **Incubate 30 minutes on ice.**

   These conditions will usually sufficiently permeabilize the outer membrane of *E. coli* to release the soluble periplasmic components and leave the spheroplasts intact to ensure low contamination of the protein preparation with cytoplasmic proteins. Harsher treatments, e.g. osmotic shock or use of lysozyme may be used if the periplasmic components are not completely released with the EDTA treatment alone.

4. **Collect a 10 µl sample for analysis of the total protein content via SDS-PAGE and/or Western blotting**

   The 10 µl sample should be thoroughly mixed with 90 µl Buffer W and 25 µl 5 x SDS-PAGE sample buffer. Store at -20°C. The whole sample must be incubated in an ultrasonic bath for 15 minutes to break up (disrupt) chromosomal DNA and should be heated to 70°C for 10 minutes prior to SDS-PAGE.

5. **Remove spheroplasts by centrifugation at 13000 rpm (microfuge) for 5 minutes at 4°C.**

6. **Carefully transfer the clear supernatant in a clean tube.**

   To check whether a part of the expressed protein remained in the cells, resuspend the sedimented spheroplasts with 1 ml Buffer P and add 250 µl 5x SDS-PAGE sample buffer and perform SDS-PAGE, optionally followed by Western blotting to detect the recombinant protein specifically.
   Store the supernatant on ice until chromatography or store at -20°C if chromatography cannot be performed the same day.

3 Purification of *Strep*-tag fusion proteins using *Strep*-Tactin Spin Columns

Material and important notes

- The spin column matrix binds up to 4 nmol recombinant *Strep*-tag fusion protein (corresponding to 150 µg of a 37 kDa protein (GAPDH-*Strep*-tag))
- Buffer W (washing buffer): 100 mM Tris·Cl, 150 mM NaCl, 1 mM EDTA, pH 8
- Buffer BE (elution buffer): 100 mM Tris·Cl, 150 mM NaCl, 1 mM EDTA, 2 mM D-biotin, pH 8
- The composition of the lysis, wash and elution buffers can be modified to suit the particular application, e.g. by adding a mild detergent, a reducing reagent, protease inhibitors, glycerol or by modifying the ionic strength. The pH should not be lower than 7.5, though. For more information see Table 1 on page 3
- Generally, it is recommended to perform protein purification at 4°C

1. Centrifuge cleared lysates (13,000 rpm, 5 minutes, 4°C, microfuge).

Insoluble aggregates which may have formed after storage may clog the column and thus have to be removed.

2. Equilibrate the Strep-Tactin Spin Column with 2x 500 µl Buffer W. Centrifuge at each step for 30 seconds at 700 x g (approx. 2000 rpm). Discard the flow-through.

This rehydrates the dried *Strep*-Tactin resin for the subsequent use. The spin column should be loaded with the cleared lysate containing *Strep*-tag proteins within 20 min, otherwise the capacity of the column might decrease.
Use buffer without EDTA for metalloproteins.

3. Load up to 500 µl supernatant of cleared lysate onto the pre-equilibrated *Strep*-Tactin Spin Column. Centrifuge for 30 seconds at 700 x g (approx. 2000 rpm).

Collect the flow-through. Apply 2 µl to an analytical SDS-PAGE.
Lysates with the recombinant protein at low concentration may lead to reduced yields and should be concentrated prior to chromatography. If quantification is possible, apply a volume of lysate containing between 3 and 5 nmol recombinant *Strep*-tag II fusion protein.
For very concentrated cell lysates, it may be necessary to extend the centrifugation time to 3-4 minutes.

4. Wash the column 4 times with 100 µl Buffer W. Centrifuge at each step for 30 seconds at 13,000 rpm.
Collect the flow-through. Apply 2 µl of the first washing fraction and 20 µl of each subsequent fraction to an analytical SDS-PAGE.

5. **Place the spin column into a fresh 1.5 ml reaction tube and choose one of the following procedures for elution:**

   a. **For maximum protein yield:**
   Elute the recombinant protein by adding 3 times 150µl Buffer BE (Biotin-Elution-Buffer). At each step: First, centrifuge for 30 seconds at 700 x g (approx. 2000 rpm) and finish with 15 seconds at maximum speed. Pool the eluates.

   b. **For maximum protein concentration:**
   Elute the protein with 50µl Buffer BE (Biotin-Elution-Buffer). First, centrifuge for 30 seconds at 700 x g (approx. 2000 rpm) and finish with 15 seconds at maximum speed. Transfer the eluate from the collection tube onto the spin column and repeat the centrifugation step as above to maximize the yield.

10 µl samples of each fraction can be used for SDS-PAGE analysis.
D-biotin and EDTA can be removed, if necessary, via dialysis or gel chromatography.
Strep-Tactin spin columns cannot be re-used.

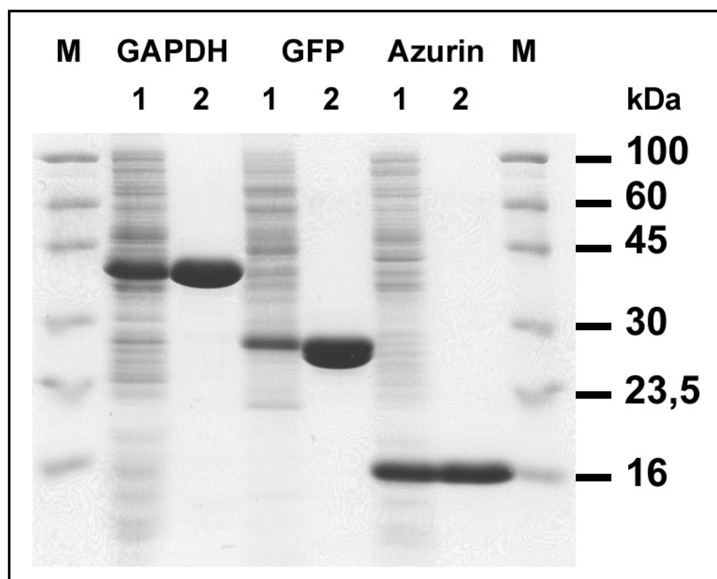

Figure 1: Three different *Strep*-tag proteins were expressed in *E. coli*, purified from 10 ml culture using *Strep*-Tactin spin columns, and eluted with 100 µl Buffer BE (Biotin Elution Buffer). 8 µl of each eluate were loaded on a SDS-PAGE. Proteins were visualized by Coomassie staining.

1: cell lysate
2: eluate

Trouble shooting

Problem: No or weak binding to *Strep*-Tactin column	
pH is not correct.	The pH should be > 7.5
Strep-tag II is not present.	Use protease deficient *E. coli* expression strains. Add protease inhibitors during cell lysis.
Strep-tag II is not accessible.	Fuse *Strep*-tag with the other protein terminus; use other linker.
Strep-tag II has been degraded.	Check that the *Strep*-tag is not associated with a portion of the protein that is processed.
Strep-tag II is partially accessible.	Reduce washing volume to 2 times 100 μl.
Strep-Tactin column is inactivated by biotin.	Add avidin if biotin containing extracts are intended to be purified (The total biotin content of the soluble part of the total E. coli cell lysate is about 1 nmol per liter culture (OD_{550} = 1.0), however, it might be much higher when proteins are purified from cell culture supernatants. Add 2-3 nmol of avidin monomer per nmol of biotin.
Problem: Contaminating proteins	
Contaminants derive from remaining lysate.	Due to the spin column design it might happen that small amounts of lysate or washing buffer remain on the plastic ring fixing the column. Removal of such liquid prior to next step will achieve highest purities. Elution buffer should be applied in the center of the column.
Contaminants are short forms of the tagged protein.	Use protease deficient *E. coli* expression strains. Add protease inhibitors after cell lysis. Fuse the *Strep*-tag II with the other protein terminus. Check for the presence of internal translation initiation starts (only in case of C- terminal *Strep*-tag II) or premature termination sites (only in case of N- terminal *Strep*-tag II). Add 6xHis-tag to the other terminus and use both tags for purification which will lead to full length protein preparations.
Contaminants are covalently linked to the recombinant protein via disulfide bonds.	Add reducing reagents to all buffers for cell lysis and chromatography.
Contaminants are non-covalently linked to the recombinant protein:	Increase ionic strength in all buffers for cell lysis and chromatography (up to 5 M NaCl) or add mild detergents (up to 2 % Triton X-100, 2 % Tween, 0.1 % CHAPS, etc).

Note: The soluble part of the *E. coli* total cell extract contains no proteins beyond the nearly irreversibly binding biotin carboxyl carrier protein (BCCP) which binds significantly to the *Strep*-Tactin column. Therefore, contaminating proteins interact, specifically or non-specifically, with the recombinant protein itself and, therefore, are co-purified.

Patents & Licensing

IBA patents, licensing and trademarks

Strep-tag® technology for protein purification and detection is covered by US patent 5,506,121, UK patent 2272698 and French patent 93 13 066; the tetracycline promoter based expression system is covered by US patent 5,849,576 and Strep-Tactin® is covered by US patent 6,103,493. Further patent applications are pending world-wide. Purchase of reagents related to these technologies from IBA provides a license for non-profit and in-house research use only. Expression or purification or other applications of above mentioned technologies for commercial use require a separate license from IBA. A license may be granted by IBA on a case-by-case basis, and is entirely at IBA's discretion. Please contact IBA for further information on licenses for commercial use.

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